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Chapter 7

IONTOPHORETIC DELIVERY OF APOMORPHINE: *IN VITRO* OPTIMIZATION AND VALIDATION.

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Pharm. Res. 14: 1797-1802 (1997)

Abstract

Purpose. To investigate the feasibility of transdermal iontophoretic delivery of apomorphine in patients with Parkinson's disease, transdermal transport rates were optimized and validated across human stratum corneum and freshly dermatomed human skin *in vitro*.

Methods. In all experiments R-apomorphine hydrochloride was applied in the anodal compartment. The effect on the flux of the following parameters was studied, using a flow through transport cell: current density, pH, concentration, ionic strength, osmolarity, buffer strength, temperature and skin type.

Results. Transdermal transport of apomorphine was directly controlled by the presence or absence of current. Passive delivery was minimal and no depot effect was observed. A linear relationship was found between current density and steady state flux. At room temperature the lag time was 30 to 40 minutes. A maximal steady state flux was obtained when the donor concentration approached maximum solubility. By increasing the temperature of the acceptor chamber to 37 °C, the steady state flux was increased by a factor of 2.3 and the lag time decreased to ± 3 minutes. No effect of osmolarity and buffer strength, and only a small effect of ionic strength and pH, on the transport rate were observed. The flux through dermatomed human skin was decreased compared to stratum corneum. This effect was shown not to be caused by skin metabolism.

Conclusions. The results obtained *in vitro* indicate that the iontophoretic delivery of apomorphine can be accurately controlled and manipulated by the applied current. The *in vitro* flux furthermore depends on the donor composition, temperature and skin type. Under optimized conditions, transport rates resulting in therapeutically effective plasma concentrations are feasible, assuming a one to one *in vitro/in vivo* correlation.

Introduction

The choice of the delivery route and the drug delivery system should be based on a detailed knowledge of both the pharmacokinetics (PK) and the pharmacodynamics (PD) of the drug and in particular the interrelationship between the two (Danhof *et al.* 1993). The dopamine agonist apomorphine has been shown to be a very potent drug for the treatment of patients with idiopathic Parkinson's disease (IPD). Furthermore, it has proven to be particularly effective in the treatment of on-off phenomena, appearing at a later stage of the disease (Hardie *et al.* 1984). However, the complex PK/PD and pharmacokinetic/toxicodynamic relationship of apomorphine (Van Laar *et al.* 1998), necessitate accurate control over the drug input rate into the systemic circulation. This prerequisite puts high demands on the delivery system by which the drug is administered and has prevented its widespread use as an anti-Parkinson drug.

Intravenous infusion would be ideal, but is inapplicable for chronic treatment of the disease on an out-patient basis. Subcutaneous administration results in a rapid uptake into the vascular system and is currently the method of choice for long-term administration (Lees 1993). A significant relief from the symptoms of Parkinson's disease and a reduction of systemic side effects was obtained, when the input rate was titrated to the individual patient's need (Frankel *et al.* 1990). However, a dose related appearance of subcutaneous nodules is a recurrent problem for which no solution has been found until now (Lees 1993).

In vivo attempts to deliver apomorphine through the skin by passive diffusion are known. Gancher *et al.* did not succeed in achieving detectable plasma levels in patients with Parkinson's disease (Gancher *et al.* 1991). In rabbits apomorphine was found in the blood upon passive delivery from a hydroxy propyl methylcellulose gel (Durif *et al.* 1994). The surface area needed to attain the necessary input rate will determine whether similar results can be obtained in humans.

From *in vitro* studies it is known for a variety of drugs, that accurate external control of the input rate, as well as a significant increase over passive transdermal delivery can be achieved by iontophoresis (Sage 1995, Yoshida *et al.* 1992). By simple adjustment of the current density, a proportional increase or decrease of the drug input rate could be obtained. As identified by many investigators, and proven for the many drugs that have been applied by iontophoresis *in vitro*, multiple formulation parameters such as concentration, pH and ion composition, may influence the flux (Green 1996). Apart from these parameters, validation of the *in vitro* experimental parameters is of importance when optimized formulations are to be applied in patients. Furthermore, by varying these parameters, determinants of the transport rate *in vivo* may be identified.

The aim of this study was to identify the variables that determine the flux and to optimize the iontophoretic flux of apomorphine across human skin *in vitro*. The dependence of transdermal transport on current and the effect of several donor components such as drug concentration, pH, osmolality and ionic composition were studied for this. In order to validate these findings, additional experiments were performed investigating the importance of metabolism, temperature and skin type. The importance of skin metabolism was also determined. Furthermore, the effects of experimental parameters (e.g. temperature and skin type) were studied.

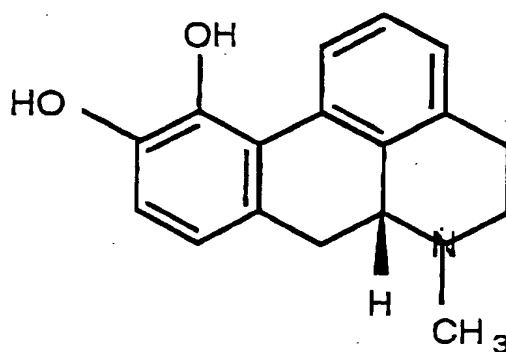
Materials and Methods

Materials

R-Apomorphine hydrochloride was obtained from OPG (Utrecht, The Netherlands). Purity was tested by high performance chromatography (HPLC) on a chiral column and found to be > 99 %. Silver and silver chloride were obtained from Aldrich (Bornem, Belgium) and were > 99.99 % pure. All other chemicals used were of the highest obtainable purity. HPLC grade acetonitrile (Rathburn, Walkerburn, Scotland) was used as a solvent in the HPLC analysis.

Dialysis membrane with a cut-off of 10.000 from Diachema (München, Germany) was used as a support membrane. Human skin samples were obtained by surgical removal from female donors. After removal of residual fat, skin was dermatomed at $\pm 200 \mu\text{m}$ using a Padgett Electro Dermatome Model B (Kansas City, USA). It was used directly when full skin experiments were performed. To obtain stratum corneum, skin was dermatomed within 24 hours after surgical

removal and incubated with its dermal side down on Whatman paper, soaked in a solution of 0.1 % (w/w) trypsin in 0.15 M phosphate buffered saline (PBS: NaCl 8 g.l⁻¹, Na₂HPO₄ 2.86 g.l⁻¹, KH₂PO₄ 0.2 g.l⁻¹, KCl 0.19 g.l⁻¹, pH 7.4) for 24 hours at 4 °C and subsequently for 1 hour at 37 °C. After this the stratum corneum can be peeled off from the underlying epidermis and dermis. Remaining trypsin activity was blocked by bathing the stratum corneum in a 0.1 % (w/w) trypsin inhibitor solution (type II-S from soybean, Sigma Chemicals, Zwijndrecht, The Netherlands) in PBS. The stratum corneum was subsequently washed several times in doubly distilled water and stored in a silica gel containing desiccator in a N₂ environment to inhibit oxidation of the stratum corneum lipids. Stratum corneum of less than 4 months old was used in the experiments.



Mol. weight (apomorphine-HCl): 312
pK_a's: 7.2, 8.9
log P_{oct/water}: 2.15

figure 1. Structure and physicochemical parameters of apomorphine.

Diffusion cell and procedures

A three chamber continuous flow through diffusion cell was used for the permeation experiments (Van der Geest *et al.* 1998a). Stratum corneum (Ø 14 mm) was hydrated for two hours by floating the dermal side on PBS. Dialysis membrane was used as a support membrane (boiled for 30 minutes in doubly

distilled water prior to use). Dermatomed skin (\varnothing 16 mm) was used directly and supported by a piece of Whatman paper of the same diameter. One membrane was then clamped between each outer chamber and the middle chamber. The two skin membranes separate the outer electrode chambers from the middle acceptor chamber, facing the electrodes with their anatomical surfaces. The exposed areas were 0.64 cm^2 , the acceptor volume 0.5 ml and the donor volume 2 ml. To mimic physiological circumstances the acceptor chamber, but not the two outer chambers, could be thermostated at 37°C , by a surrounding socket. Unless stated otherwise, the flux experiments were conducted at room temperature. After assembling the system, the acceptor chamber was flushed for 30 minutes. PBS was used as acceptor fluid (flow rate: 6.5 ml.h^{-1}) and in the cathodal chamber. To maintain viability 1 g.l^{-1} glucose was added to the PBS in the dermatomed skin experiments. In all experiments the donor chamber contained a citrate buffered apomorphine hydrochloride solution ($\text{NaCl } 8.18 \text{ g.l}^{-1}$, 5 mM citrate buffer: citric acid/ $\text{Na}_3\text{-citrate} = 0.62/0.63$, $0.37/0.96$, $0.12/1.30 \text{ g.l}^{-1}$ for pH 4,5,6, respectively). The structure and physico-chemical parameters of apomorphine are given in fig. 1. At the donor pH's used in this study apomorphine is a cation with a charge of 1. The drug was therefore applied in the anodal chamber at all conditions tested. During the experiments both the anodal and the cathodal chambers were stirred continuously at 375 rpm.

Precautions were taken to prevent auto-oxidative breakdown of apomorphine: 1) a citrate buffer was used in the donor compartment (binds divalent cations); 2) 0.1 % sodium meta bisulphite (anti-oxidant) was used in donor and acceptor phases; 3) 100 μl of a solution of 0.5 % sodium meta bisulphite, 0.05 % EDTA in 25 % conc. phosphoric acid was applied in the collecting tubes and 4) collection tubes, and HPLC samples were shielded from light.

Breakdown of apomorphine was checked visually (a breakdown of approx. 1 % in the donor results in green or yellow coloring, depending on the vehicle composition) and by HPLC analysis. After having taken the proper precautions no breakdown of apomorphine was observed. EDTA was omitted in the diffusion studies since histopathological investigations following subcutaneous application of EDTA containing apomorphine formulations in patients, provided evidence of inflammation due to the presence of EDTA (Van Laar *et al.* 1997). After each experiment, the pH of the donor phases was checked. If a pH shift of more than 5 % was observed the data of that particular cell were discarded.

Iontophoretic protocol

A silver plate electrode was used in the anodal compartment and a silver/silver chloride electrode was used in the cathodal compartment. The electrodes were connected to a 9-channel computer controlled current source that was able to deliver both constant and constant-pulsed current of variable frequency and duty cycle. The system was equipped with two differential input channels per current source channel, enabling on-line resistance measurements of the individual membranes of each transport cell. The maximum voltage for each channel was 40 Volts. The current source was custom made at the electronics department of the Gorlaeus Laboratories (Leiden, The Netherlands).

Typically, current was applied for five or six hours. Resistance was measured during current application at 30 or 60 seconds intervals. In some cases, samples were collected after termination of the current to analyze passive transport.

The following protocols were tested ($n = 3 - 4$):

<i>protocol</i>	<i>I</i> ($\mu\text{A}.\text{cm}^{-2}$)	<i>temp</i> ($^{\circ}\text{C}$)	<i>donor conc.</i> (mM)	<i>pH</i>
I	0	20	15	5
II	500	20	15/0&15-post ¹	5
III	100/200/300/500	20	15	4
IV	500	20	15	4/5/6
V	500	20	1/5/10/15	5
VI	500	37	15	5

¹ Current passage without drug, followed by passive diffusion with 15 mM apomorphine

Sample analysis

For the analysis of apomorphine and its metabolites apocodeine and isoapocodeine the method of Van der Geest *et al.* (1997) was used with some modifications. Briefly the method was as follows: 20 :1 samples were injected directly into the HPLC system consisting of a Spectroflow 400 solvent delivery system (Applied Biosystems, Ramsey, NJ, USA), a Promis autosampler (Spark Holland B.V., Emmen, The Netherlands) and a fluorescence detector (Jasco 821-FP, H.I. Ambacht, The Netherlands). The excitation wavelength was 280 nm, the emission wavelength was 460 nm. A nucleosil 100, 5- μm C-18 column was used (200 mm x 4.6 mm I.D.) (YMC, Morris Planes/NJ, USA). The mobile phase consisted of acetonitrile/aqueous phase (25:75 v/v). The aqueous phase contained 0.1 M NaH_2PO_4 adjusted to pH 3 with phosphoric acid, 20 $\text{mg}.\text{ml}^{-1}$ 1-

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octanesulfonic acid (Sigma, Bornem, Belgium) and 10 mg.ml⁻¹ EDTA. The flow rate was 0.75 ml.min⁻¹. Chromatography was performed at room temperature. The calibration curves for all three compounds were linear ($r > 0.999$) in the concentration range of 10 - 1000 ng.ml⁻¹. The intra- and inter-assay variations were $< 5 \%$ for all concentrations tested. The detection limit under these conditions was 50 fmol.

For the analysis of glucuronidated and sulphated apomorphine a deconjugation reaction and a subsequent extraction of apomorphine was performed prior to quantification on the HPLC system (Van der Geest *et al.* 1997).

Data analysis

Apomorphine fluxes were calculated from the concentrations in the tubes taking into account acceptor flow rates, area of diffusion, sample intervals and dilution steps using the basic equation,

$$J_{app} = F \cdot C / A \quad (1)$$

where J_{app} is the apparent flux through the membrane, C is the acceptor concentration, A the area of the skin available for diffusion and F is the flow rate (Van der Geest *et al.* 1998a).

The lag time was defined as the intercept of the linear part of the cumulatively plotted flux versus time and the time axis. A t-test was used to test for significant changes of the steady state flux compared to control values.

Statistical analysis

All results are the means of 3 to 4 experiments and data are presented as mean \pm SD. Statistical differences are tested using a Student's *t* test. Statistical significance was defined as $p < 0.05$.

Results

Flux optimization experiments

As can be observed in figure 2, no appreciable amounts of apomorphine were delivered by passive diffusion when protocol I was applied. The application of an iontophoretic current according to protocol II, greatly enhanced the delivery of apomorphine across human stratum corneum to a steady state flux of 90 ± 6 nmol.cm⁻².h⁻¹ (fig. 2). A steady state flux was attained in approximately three

hours, corresponding with a lag time of 40 minutes. Reversibility of the enhanced membrane permeability to the 'normal' state was tested by measuring the passive drug flux after switching off the current following the five hour period of iontophoretic transport at the highest current density applied ($500 \mu\text{A}.\text{cm}^{-2}$). In a period of only 3 hours, the flux had decreased exponentially to a value approximately 1/20-th the steady state iontophoretic flux. This value was still significantly elevated compared to the passive flux of apomorphine. In a control experiment, stratum corneum was exposed to the same anodal current density for five hours without the co-application of apomorphine. Thereafter, passive diffusion of apomorphine was studied for several hours. A significantly enhanced passive diffusion was observed due to current treatment alone. This value is still lower than the post-iontophoretic passive value, which is probably due to the fact that steady state has not been reached after 3 hours.

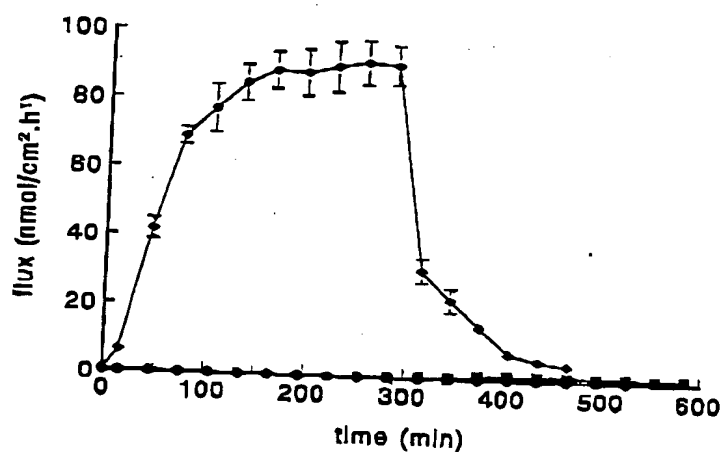


Figure 2. Iontophoretic and passive flux versus time profile of apomorphine through human stratum corneum at a 15 mM donor concentration, pH 5 at 20 °C. (1) five hours of iontophoretic delivery at $500 \mu\text{A}.\text{cm}^{-2}$ followed by three hours of passive delivery ($-\circ-$), (2) passive flux after five hours of current pretreatment ($500 \mu\text{A}.\text{cm}^{-2}$) without apomorphine ($-\blacksquare-$), (3) passive flux through non-pretreated skin ($-\bullet-$).

The relationship between current density and steady state flux was determined at a 15 mM donor concentration at pH 4. As shown in protocol III the current density range 0 to 500 $\mu\text{A}\cdot\text{cm}^{-2}$ was studied. A linear relationship ($r^2 = 0.98$) between current density and steady state flux was found (fig. 3). For all current densities tested, the time to reach a steady state flux was less than two hours.

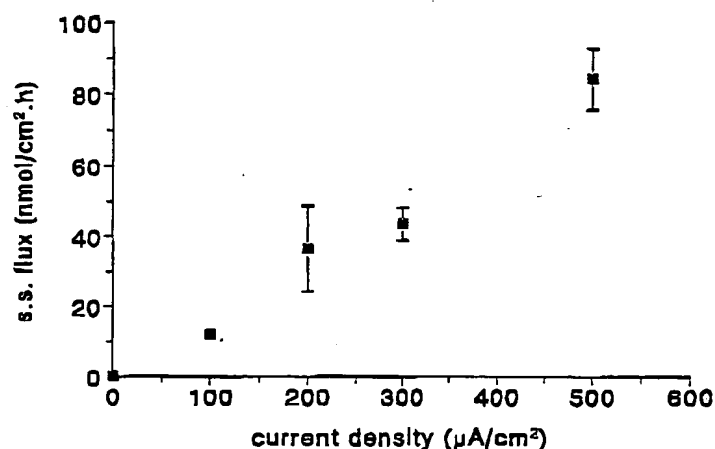


figure 3. Relationship between the applied current density and the steady state flux of apomorphine through human stratum corneum at a 15 mM donor concentration, pH 4 at 20 °C (current density range is 100 - 500 $\mu\text{A}\cdot\text{cm}^{-2}$).

A slight, but not significant increase in the mean steady state resistance of about 20% was observed when the donor pH was increased from 4 to 5 (protocol IV). No further increase was observed when the pH was raised to pH 6 (table 1). The steady state flux at pH 5 was slightly increased compared to the previous value obtained when protocol II was applied. This difference can be ascribed to the fact that stratum corneum was obtained from a different donor. At pH 5 the effect of buffer strength was also investigated (data not shown). The buffer strength was increased two-fold, however no effect on the flux was observed in this case. Donor formulations with pH > 6 were not tested due to decreased stability of apomorphine under such conditions.

Table 1. Effect of pH on steady state flux (J_{ss}) through human stratum corneum (donor concentration 15 mM, current density $500 \mu A.cm^{-2}$, $20^\circ C$, $n = 4$).

pH	mean J_{ss} ($nmol.cm^{-2}.h^{-1}$)
4	84.2 ± 8.6
5	98.4 ± 8.8
6	100.4 ± 9.3

The steady state flux of apomorphine increased with increasing donor concentration at pH 5 (protocol V, fig. 4). The relationship of the steady state flux versus donor concentration leveled off at concentrations that approached maximum solubility (± 60 mM). An increase of the steady state stratum corneum resistance was observed with increasing apomorphine concentrations (Table 2).

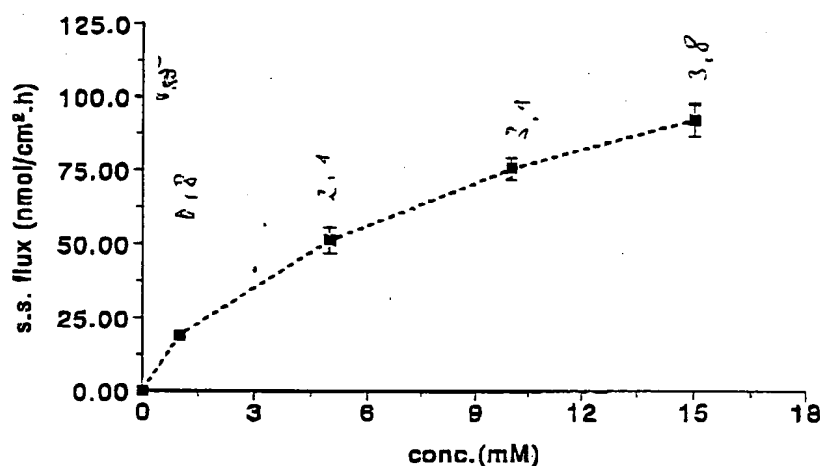


Figure 4. Relationship between the donor concentration of apomorphine and the steady state flux through human stratum corneum at a current density of $500 \mu A.cm^{-2}$, at $20^\circ C$ (donor concentration range is 0 - 15 mM at pH 5).

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Table 2. Effect of concentration on the total steady state resistance(R_{ss}) that was measured current density (pH 5, 500 $\mu\text{A}.\text{cm}^{-2}$, 20 °C).

conc. (mM)	R_{ss} (kOhm)
0	6.0 ± 1.5
1	6.4 ± 0.8
5	11.6 ± 1.5
10	23.1 ± 7.3
15	26.6 ± 3.0

The effects of ionic strength and osmolarity were studied at pH 5, 15 mM donor concentration and a current density of 500 $\mu\text{A}.\text{cm}^{-2}$. Lowering the NaCl concentration in the donor chamber from 150 to 35 mM resulted in a 30 % increase of the steady state flux. When the osmolarity of the acceptor phase was increased by dissolving 0.1 M mannitol, no effect was observed. Neither the steady state flux nor the lag time of apomorphine transport through the stratum corneum changed compared to standard conditions (protocol II, 15 mM donor conc.). Skin samples of multiple donors have been tested under these standard conditions. The intra -and inter subject variability was never more than 10 percent.

Validation experiments

Changes in flux kinetics through stratum corneum were observed when the transport experiment was executed at physiological temperature instead of room temperature (protocol VI). The steady state flux was increased 2.3 times whereas the lag time decreased to 3 minutes (fig. 5).

The transport through 200 μm dermatomed full skin was also studied at physiological temperature. Using protocol VI, changes in transport kinetics again occurred: The steady state flux decreased significantly by 30 % compared to the flux through stratum corneum at 37 °C. However the lag time increased to 130 minutes (fig. 6).

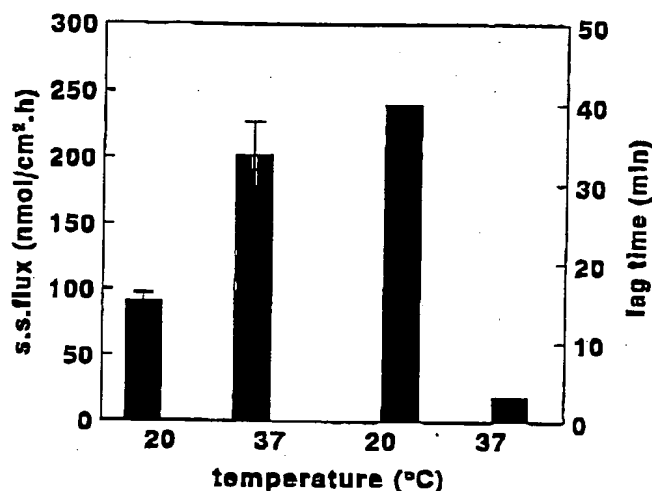


figure 5. Histogram of the temperature dependence of apomorphine transport through human stratum corneum at a current density of $500 \mu\text{A}.\text{cm}^{-2}$, and a 15 mM donor concentration pH 5. Steady state flux (left Y-axis) and lag time (right Y-axis) were determined at 20 and 37 °C.

The collected samples of the full skin experiment were tested for the possible formation of metabolites. The half hour samples collected at two and six hours were analyzed. No COMT metabolites (apocodeine, iso-apocodeine) could be detected by direct analysis on the HPLC system. No significantly increased concentrations of apomorphine were found when the samples had been incubated with deconjugation enzymes (β -glucuronidase and sulphatase) for five hours. From this result it was concluded that glucuronidated and sulphated apomorphine were not formed in significant amounts during iontophoretic transport through skin that was dermatomed at 200 μm .

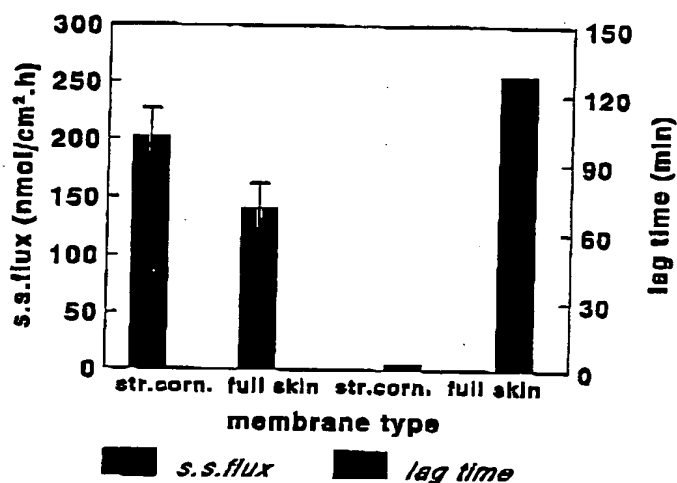


figure 6. Histogram of the membrane dependence of apomorphine transport at 37 °C, a current density of 500 $\mu\text{A}.\text{cm}^{-2}$, and a 15 mM donor concentration pH 5. Steady state flux (left Y-axis) and lag time (right Y-axis) were determined through human stratum corneum and 200 μm dermatomed full skin.

Discussion

In this study it was shown that *in vitro* the transdermal transport of apomorphine by iontophoresis can be greatly enhanced over passive delivery, as is to be expected from a (positively) charged drug. It was also shown that the passive flux after iontophoretic transport returns to a level that is comparable to the passive flux of skin that was pretreated with current. It can be concluded that the barrier properties remain largely intact in both cases and that no depot effect was observed for apomorphine. Other investigators have also studied the passive flux after iontophoresis *in vitro*. In most cases it was found that the flux declines rapidly after the current was switched off, resulting in a passive level that is usually somewhat higher than the passive flux through untreated skin (Green *et al.* 1991, Volpato *et al.* 1995). In some cases the flux retained a level comparable to iontophoretic transport (Préat *et al.* 1993). Enhanced passive levels have often been attributed to an alteration of the barrier as a result of current application.

However, other indirect effects might also be important: Increased water content, induced by iontophoresis, may lead to increased flux of apomorphine through the skin. For CQA 206-291, a drug with physicochemical properties comparable to apomorphine, a depot formation was observed only, when a saturated alcohol/water solution of the drug was applied to the skin (Hager *et al.* 1993). The flux was shown to be linearly proportional to the applied current density. This is found for almost all compounds, if applied by iontophoresis. This, together with the small inter- and intra-donor variability in the transport rate of apomorphine, makes it possible to externally control the input rate of apomorphine. Easy manipulation of the resulting plasma concentration equally depends on the pharmacokinetic characteristics of the drug. The high clearance of apomorphine and short terminal half life make it possible to rapidly change the plasma concentration if needed (Van der Geest *et al.* 1998b).

It was observed that the permeability of the stratum corneum decreases with increasing donor concentration of apomorphine: The steady state flux apparently reaches a plateau towards the maximal solubility of apomorphine in the donor compartment. This phenomenon was previously observed for a number of other molecules including hydromorphone (Padmanabhan *et al.* 1990) and a number of positively charged lipophilic peptides (Delgado-Charro *et al.* 1994, Hoogstraate *et al.* 1994). For these peptides it was shown that iontophoresis of these compounds can cause a change in direction and/or magnitude of the electro-osmotic flow, that can lower their transport efficiency. Increasing the donor concentration of apomorphine also results in an increase of the skin resistance. Additional experiments have shown that the skin resistance returns to commonly observed values ($< 5 \text{ k}\Omega\text{m}$) when the apomorphine formulation is replaced by the vehicle only. The transport efficiency of apomorphine is less than 1 %, therefore the major part of the current is carried by small ions. The electric properties of the skin are therefore predominantly determined by these ions. Apomorphine apparently decreases the accessibility of the available pathways for small ionic species. The mechanism of this effect is currently unknown.

Various strategies for optimization of iontophoretic transport have been suggested (Sanderson *et al.* 1989, Wearley *et al.* 1989). For apomorphine only small effects of pH and ionic strength were observed. The small increase in steady state flux of the positively charged apomorphine, when the pH was raised to 5, is likely to be caused by an increase of the net negative charge of the skin. The increase is not believed to be caused by a change in competitive ion concentration: $[\text{H}^+]$ decreases from 0.1 to 0.01 mM. Also the buffer strength of the citrate buffer is kept very low (5 mM). Therefore changes in competitive ion

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concentrations are believed to be negligible compared to the sodium ion concentration (150 mM). Further optimization of iontophoretic transport through a lowering of the NaCl concentration can only be applied *in vivo* when depletion of ions is prevented. If not, pH shifts may occur that can result in adverse skin effects.

The results show that the effectivity of optimization strategies depends on the investigated drug. Enhancement by the co-application of alcohol has little chance of success: This strategy was tested for the iontophoretic delivery of CQA 206-291 (Hager *et al.* 1993), a dopamine agonist with comparable physical-chemical and structural characteristics to apomorphine. No additional enhancement was obtained. We found a significant increase of the steady state flux when the acceptor phase temperature was raised from room temperature (20 °C) to physiological values (37 °C). This temperature dependent enhancement of transport suggests an involvement of the intercellular pathway, by a fluidization of the lipids in the intercellular domains. This opens up the possibility of optimizing the transport by the application of enhancers that are known to increase the fluidity of the lipid bilayers. For other compounds a more hydrophilic porous pathway has shown to be most probable (Phipps *et al.* 1992). Due to the limited level to which the skin temperature can be raised *in vivo* and the instability of apomorphine at higher temperatures, the observed effect has little practical applicability as an enhancement strategy in this case.

A decrease of the steady state flux and an increase of the lag time were observed when stratum corneum was replaced by freshly dermatomed full skin. Since no metabolism was observed during iontophoretic transport through this membrane, the observed differences are likely to result from additional barrier properties of the epidermis and dermis. The increase in lag time is surprising, since drugs are generally known to rapidly diffuse through the dermis. It may indicate that apomorphine slowly accumulates in the dermal region. This may be caused by the absence of dermal clearance in the *in vitro* situation and may therefore not be observed *in vivo*. It is known that the dermal clearance can substantially influence the transdermal transport *in vivo* (Patel *et al.* 1974).

Assuming a 1 to 1 correlation between the steady state flux *in vivo* and the validated steady state iontophoretic flux *in vitro* the necessary transport area can be calculated, by applying equation $J_{app} / A = Cl * C_{ss}$. J is the steady state flux, A is the transport area, Cl is the intrinsic clearance and C_{ss} is the therapeutic steady state plasma concentration. In previous studies, in which apomorphine was applied by intravenous infusion in patients with Parkinson's disease, it was found that the clearance was $40.4 \pm 14.9 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ (Van der Geest *et al.* 1998b) and that

therapeutic plasma concentrations ranged between 3 and 6 ng.ml⁻¹ (Van Laar *et al.* 1998). When these values and the validated steady state flux are substituted in the equation it was calculated that the transport area was around 20 cm². Patches of this size can be clinically applied.

Overall it can be concluded that for the selection and optimization of an iontophoretic drug delivery system multiple considerations need to be taken into account: For apomorphine, the fact that there is considerable inter- and intra-patient variability in both the disease state and the response to apomorphine (Van Laar *et al.* 1998) will ultimately call for an individualized and adjustable input profile. This study shows that this can be achieved by transdermal iontophoresis of the drug at therapeutically significant transport rates. Iontophoretic delivery of apomorphine is therefore a promising strategy for the treatment of patients with Parkinson's disease.

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